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Analysis of Sertraline in Postmortem Fluids and Tissues in 11 Aviation Accident Victims

Russell J. Lewis
Mike K. Angier
Kelly S. Williamson

Civil Aerospace Medical Institute
Federal Aviation Administration
Oklahoma City, OK 73125

Robert D. Johnson

Tarrant County Medical Examiner's Office
Fort Worth, Texas 76196

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16. Abstract

Sertraline (Zoloft®) is a selective serotonin reuptake inhibitor that is a commonly prescribed drug for the treatment of depression, as well as obsessive-compulsive disorder, panic disorder, social anxiety disorder, premenstrual dysphoric disorder, and post-traumatic stress disorder. While the use of sertraline is relatively safe, certain side effects could negatively affect a pilot's performance and become a factor in an aviation accident. The adverse side effects associated with this medication include: sleepiness, nervousness, insomnia, headaches, tremors, and dizziness. The nature of aviation accidents often precludes the availability of blood from accident victims; therefore, tissues must be relied upon for analysis. Understanding the distribution of a drug throughout postmortem fluids and tissues is important when trying to interpret drug impairment and/or intoxication. Our laboratory investigated the distribution of sertraline and its primary metabolite, desmethylsertraline, in various postmortem tissues and fluids obtained from 11 fatal aviation accident cases between 2001-2004. The gender of the pilots was male and their ages ranged from 31 – 66. When available, 11 specimen types were analyzed for each case, including blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, heart, and bile. Human specimens were processed utilizing solid-phase extraction, followed by characterization and quantitation employing GC/MS. Whole blood sertraline concentrations obtained from these 11 cases ranged from 0.005 to 0.392 µg/mL. The distribution of sertraline, expressed as specimen/blood ratio, was as follows: urine 0.47 ± 0.39 (n=6), vitreous humor 0.02 ± 0.01 (n=4), liver 74 ± 59 (n=11), lung 67 ± 45 (n=11), kidney 7.4 ± 5 (n=11), spleen 46 ± 45 (n=10), muscle 2.1 ± 1.3 (n=8), brain 22 ± 14 (n=10), heart 9 ± 7 (n=11), and bile 36 ± 26 (n=8). Postmortem distribution coefficients obtained for sertraline had coefficient of variations (CV) ranging from 47 – 99%. With such large CV's, the distribution coefficients have very little use in aiding in the interpretation of sertraline-positive tissue specimens. Furthermore, no consistent desmethylsertraline/sertraline ratio was identified within any specimen group. This study suggests that sertraline likely undergoes significant postmortem redistribution.

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CONTENTS

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INTRODUCTION

The Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) is responsible under Department of Transportation Orders 8020.11B and 1100.2C to "conduct toxicological analysis on specimens from … aircraft accident fatalities" and "investigate … general aviation and air carrier accidents and search for biomedical and clinical causes of the accidents, including evidence of … chemical (use)." Therefore, following an aviation accident, samples are collected at autopsy and sent to CAMI's Bioaeronautical Sciences Research Laboratory, where toxicological analysis is conducted on various postmortem fluids and tissues. Occasionally during a toxicological evaluation, potentially impairing compounds are detected in postmortem specimens from aviation accident victims. The laboratory receives blood in approximately 70% of cases received from an aircraft accident; thus it relies solely on tissues for approximately 30% of the cases. Therapeutic levels of a drug are usually only reported in the scientific literature for blood or plasma. However, since blood is not available for all cases sent to CAMI's Toxicology Laboratory, it is necessary to evaluate the distribution of commonly encountered drugs.

Sertraline, also known as Zoloft® [(1S,4S)-4-(3,4-Dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphtalenamine] (Figure 1), belongs to a class of drugs called selective serotonin reuptake inhibitors (SSRIs). Other drugs in this class include Prozac® (fluoxetine), Paxil® (paroxetine), Celexa® (citalopram), and Luvox® (fluvoxamine). Sertraline is commonly prescribed to treat depression, as well as obsessive-compulsive disorder, panic disorder, social anxiety disorder, premenstrual dysphoric disorder, and post-traumatic stress disorder.[1-3] Treatment of depression with sertraline is generally safe; however, there are side effects associated with sertraline, which could specifically affect a pilot's performance. The most serious of these adverse side effects include: sleepiness, nervousness, insomnia, headaches, tremors, and dizziness.[1,3,4] For this reason, all postmortem cases received by the CAMI forensic toxicology laboratory is screened for sertraline, employing gas and liquid chromatography/mass spectroscopic techniques.

Typically, the recommended oral dose for sertraline is 25 – 200 mg once daily. Treatment usually starts at 25 – 50 mg once daily and then increases at weekly intervals until the desired response is attained. Sertraline is highly bound to plasma proteins (~98%) and is absorbed slowly from the gastrointestinal tract.[1,3-6] Following oral administration, peak plasma concentrations are achieved within 6 to 8 hours and range from 0.050 – 0.250 µg/mL (therapeutic range).[1] Less than 1% of a dose of sertraline is excreted as the unchanged parent drug in the urine.[1,2,4,7] Sertraline undergoes extensive first-pass metabolism in the liver via demethylation to form the primary metabolite, desmethylsertraline (Figure 1).

Additional hepatic drug metabolism reactions involving the biotransformation of sertraline and desmethylsertraline into Phase I/II metabolites are facilitated by oxidative deamination, hydroxylation, and glucuronidation through various CYP450 isozymes, monoamine oxidases, and glucuronyl transferase enzymes.[1,6,8-10] The metabolism pathway of sertraline and its predominant metabolites are illustrated in Figure 1.[6,8,10] The elimination half-life for sertraline is approximately 26 hours.[1,4,6,7,11-13] Desmethylsertraline has a reported elimination half-life ranging from 62 – 104 hours and has approximately 20% pharmacological activity.[3,4] The published volume of distribution (V_d) for sertraline ranges from 20 to 76 L/kg.[2,7,14-16]

Scientific information concerning the postmortem distribution of sertraline is limited to drug overdose cases. Therefore, to better understand the distribution of therapeutic, non-fatal sertraline concentrations in postmortem cases, our laboratory set out to determine its distribution in various postmortem tissues and fluids. A search of our laboratory database identified 11 aviation fatalities from 11 separate aviation accidents that were reported positive for sertraline in blood and also had most biological tissues/fluids available for analysis. These specimen types included: blood, urine, vitreous humor, muscle, liver, kidney, lung, spleen, brain, and heart. This manuscript describes a suitable method for the isolation, characterization, and quantitation of both sertraline and desmethylsertraline in postmortem fluids and tissues, as well as examines their concentrations and distribution in those fluids and tissues.

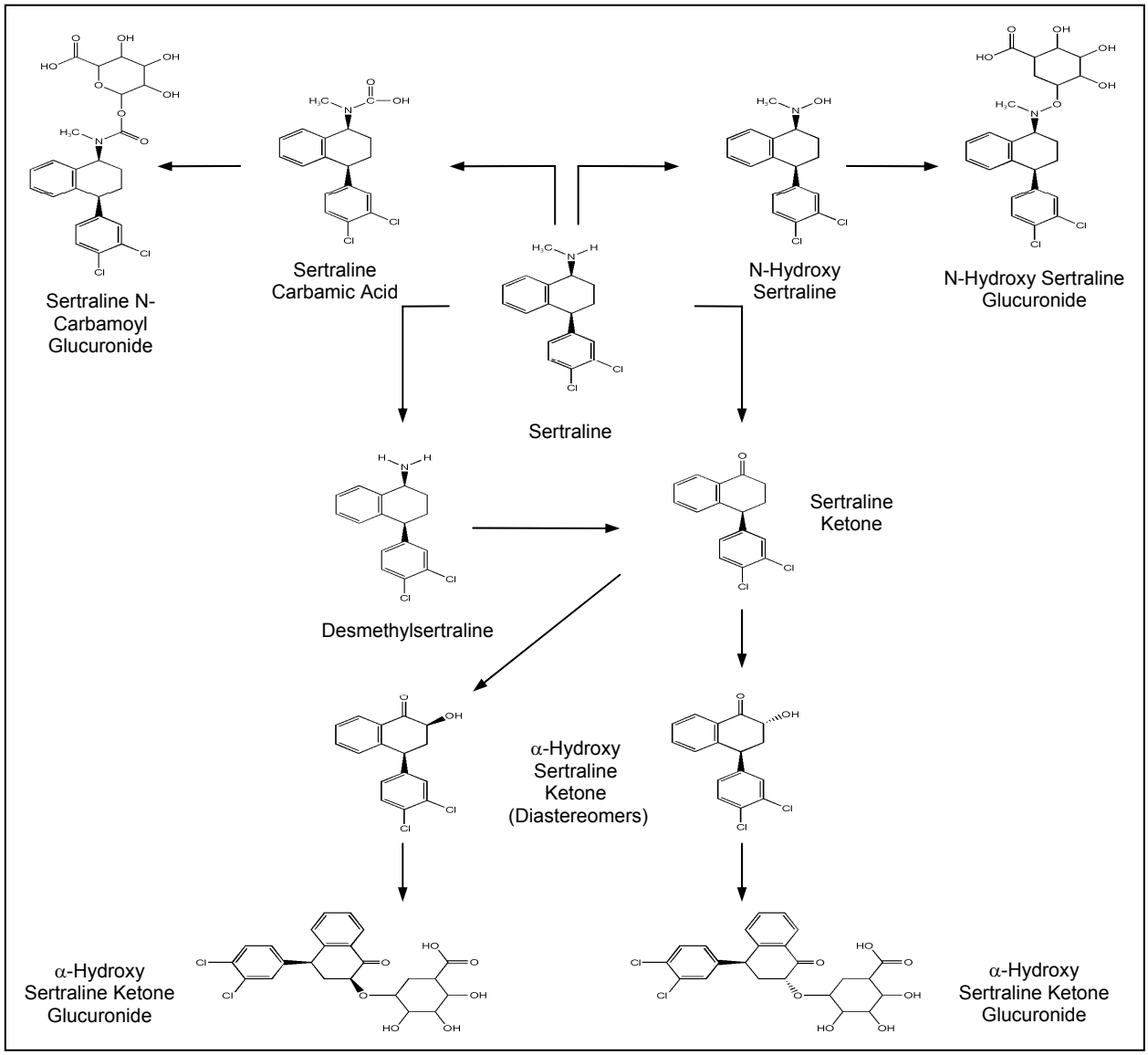

Figure 1. Human metabolic pathway of sertraline.[6,8,10]

MATERIALS AND METHODS

Chemicals and Reagents

All aqueous solutions were prepared using double deionized water (DDW), which was obtained using a Milli-QT_{plus} Ultra-Pure Reagent Water System (Millipore,® Continental Water Systems, El Paso, TX). All chemicals described below were purchased in the highest possible purity and used without any further purification. Sertraline and desmethylsertraline were purchased from Cerilliant (Cerilliant Corp., Round Rock, TX) as methanolic standards at a concentration of 1 mg/mL in sealed glass ampoules. The internal standard, SERTIS (N-Methyl-4-(4-bromophenyl)-1,2,3,4-tetrahydro-1-naphthylamine hydrochloride) was obtained from Pfizer (CP-53,630-1, Pfizer Inc., New York, NY). The derivatization reagent, pentafluoropropionic anhydride (PFPA) was obtained from Pierce (Pierce Inc., Rockford, IL). Methanol, acetonitrile, ammonium hydroxide, acetic acid, ethyl acetate, sodium fluoride, and potassium phosphate monobasic were purchased from Fisher Scientific (Pittsburgh, PA). The pH of all solutions was measured using a Corning model 430 pH meter (Corning Life Sciences, Acton, MA) connected to a Corning 3-in-1 model pH electrode.

at 250°C in the splitless mode with the purge time of 0.5 min. The oven temperature profile was established as follows: 130°C – 290°C at 30°C/min and a final hold time of 1.67 min, resulting in a total run time of 7 min. Derivatization of sertraline, desmethylsertraline, and SERTIS (int std) with PFPA was performed because each compound contained a polar functional group that could be replaced to produce a less polar compound with higher mass ions for GC/MS analysis. Initially, neat standards of each compound (1 µL of a 100 ng/µL solution) were injected individually and analyzed using the full scan mode of the GC/MS, which scanned from 50 to 600 AMU. Quantitation (quant) and qualifier (qual) ions for each analyte were then selected based on both abundance and mass-to-charge ratio (*m/z*). To increase reproducibility and reduce interference, high mass ions were selected when possible. The ions chosen were as follows: sertraline: 274 (quant), 276 and 451 (qual); desmethylsertraline: 274 (quant), 239 and 437 (qual); SERTIS: 286 (quant), 284 and 461 (qual), and are depicted in Table 1. Upon selection of unique ions, the MS was run in selected ion monitoring (SIM) mode with a dwell time of 30 msec for each recorded ion.

Table 1. Retention times and mass fragments for sertraline, desmethylsertraline, and sertis.

Analyte	Retention Time (min)	Quantitation Ions (m/z)	Qualifier Ions (m/z)
Sertraline-PFPA	5.18	274	276,451
Desmethylsertraline-PFPA	4.77	274	239,437
Sertis-PFPA	4.99	286	284, 461

Gas Chromatographic/Mass Spectroscopic Conditions

All analyses were performed using a bench-top gas chromatograph/mass spectrometer (GC/MS), which consisted of a Hewlett Packard (HP) 6890 series GC, interfaced with a HP 5973 quadrupole MS (Agilent, Palo Alto, CA). The GC/MS was operated with a transfer line temperature of 280°C and a source temperature of 250°C. The MS was tuned on a daily basis using perfluorotributylamine. The electron multiplier voltage was set at 106 eV above the tune value. Chromatographic separation was achieved using a Varian FactorFour® crosslinked 100% methyl siloxane capillary column 12 m x 0.2 mm i.d., 0.33 µm film thickness (Varian Co., Harbor City, CA). Helium was employed as the carrier gas and used at a flow rate of 1.0 mL/min. An HP 6890 autosampler was used to inject 1 µL of extract into the GC/MS. The GC was equipped with a split/splitless injection port operated

Acceptability criteria employed for analyte identification and quantitation were as follows: (1) ion ratios for a given analyte, measured as the peak area of a qualifier ion divided by the peak area of the quantitation ion, were required to be within ± 20% of the average of the ion ratios for each respective calibrator used to construct the calibration curve for that analyte; (2) each ion monitored was required to have a minimum signal-to-noise ratio (S/N) of 10; and (3) the analyte was required to have a retention time within ± 2% of the average retention time for each respective calibrator used to construct the calibration curve for that analyte. Analytes not meeting these criteria were reported as either negative or inconclusive.

Sample Selection and Storage

A search of the CAMI database identified 11 sertraline-positive fatalities from separate civil aviation accidents that had a majority of the desired biological

tissues and fluids (blood, urine, vitreous humor, bile, liver, kidney, muscle, lung, spleen, heart, and brain) available for analysis. These cases were over a 4-year period ranging from 2001-2004. In all cases, blood was stored at -20°C in tubes containing 1% (w/v) sodium fluoride/potassium oxalate until analysis. All other specimens were stored without preservation at -20°C prior to analysis. Blood sertraline and desmethylsertraline concentrations determined in this study were in agreement with those previously determined by our laboratory via this analytical method.

Calibrator and Control Preparation

Calibration curves for both sertraline and desmethylsertraline were prepared by performing serial dilutions utilizing bovine whole blood as the diluent. Calibrators were prepared from one set of original stock standard solutions, while controls were prepared in a similar manner as calibrators, using bovine whole blood as the diluent, but from a second set of unique stock solutions. Calibration curves were prepared at concentrations ranging from 0.78 – 800 ng/mL. A minimum of 6 calibrators were used to construct each calibration curve. Controls were prepared at concentrations of 80, 160, and 320 ng/mL and extracted with each batch of unknowns to verify the accuracy of the calibration curve.

Since suitable deuterated sertraline and desmethylsertraline were not available as internal standards, a structurally similar compound (SERTIS) was obtained from Pfizer, Inc., SERTIS is chemically identical to sertraline except that it contains one bromine atom instead of two chlorine atoms in its chemical structure. Based on its retention time, mass spectral characteristics and chemical fragments used for GC/MS analysis, SERTIS served as an excellent internal standard for this procedure (Figure 1, Table 1). The internal standard was prepared by diluting a SERTIS standard with deionized water to obtain a concentration of 500 ng/mL.

Quantitation was achieved via an internal standard calibration procedure. Response ratios for each compound were determined for every sample analyzed. The response ratio was calculated by dividing the area of the analyte peak by the area of the internal standard peak. Calibration curves were derived by plotting a linear regression of the analyte/internal standard response ratio versus the analyte concentration for each respective calibrator. These calibration curves were then used to determine the concentrations of each compound in the prepared controls and biological specimens.

Sample Preparation and Extraction Procedure

Postmortem specimens, calibrators, and controls were extracted in the following manner. Tissue specimens were homogenized using an Omni post-mounted homogenizer (Omni Int., Marietta, GA). The generator used with this homogenizer was 30 mm in diameter and set to rotate at 22,000 rpm. Tissues were homogenized following a 1:2 dilution with 1% NaF in DDW. Three mL aliquots of postmortem fluid, calibrator, and control, and 3 g aliquots of each tissue homogenate (1 g tissue) were transferred to individual 16 x 150 mm screw-top tubes. To each specimen, calibrator, and control, 1 mL of the internal standard mixture (500 ng) was added. Samples were vortexed briefly and allowed to stand at room temperature for 10 min. Nine mL ice-cold acetonitrile was added to each sample. The mixture was then placed on a rotary mixing wheel and mixed for 15 min by simple rotation of the wheel at 15 rpm. Centrifugation at 820×g for 5 min provided removal of cellular debris and proteins. Following centrifugation, the supernatant was transferred to clean 16 x 125 mm culture tubes and evaporated in a TurboVap® Concentration Workstation at 40°C (Caliper Life Sciences, Hopkinton, MA) under a stream of dry nitrogen to a volume of approximately 1 mL. Following acetonitrile evaporation, 4 mL 0.10 M phosphate buffer, pH 6 was added to each sample. The extracts were transferred to Bond Elute Certify® solid-phase extraction (SPE) columns obtained from Varian (Varian Co., Harbor City, CA), which had been pre-conditioned with 2 mL methanol, followed by 2 mL 0.10 M phosphate buffer, pH 6. Care was taken not to dry the column prior to extract addition. Column flow rates of 1-2 mL/min were maintained in each SPE step using a Varian 24 port Cerex® SPE processor (Varian Co., Harbor City, CA) with a nitrogen pressure of 5 psi.

Once each sample had passed through its respective column, the columns were washed with 1 mL of 1 M acetic acid then dried completely with 25 psi nitrogen for 5 min. The columns were again washed by adding 6 mL methanol to each. Following the methanol wash, the columns were again dried completely with 25 psi nitrogen for 5 min. The analytes were eluted off the columns with 3 mL of 2% ammonium hydroxide in ethyl acetate, which was prepared fresh daily. Eluents were evaporated to dryness in a TurboVap® set at 40°C under a stream of dry nitrogen. Derivatization was accomplished by adding 50 µL of ethyl acetate, followed by 50 µL of pentafluoropropionic anhydride (PFPA) to each specimen. The samples were then capped tightly, vortexed, and incubated at 70°C for 20 min. Following derivatization the tubes were allowed to cool to room temperature, and the contents were evaporated to dryness in a TurboVap® set at 40°C. Once dry, the contents of each tube were reconstituted in 50 µL of ethyl acetate and transferred to GC/MS vials for analysis.

RESULTS AND DISCUSSION

Analysis of Sertraline/Desmethylsertraline

The described procedure, utilizing SPE and GC/MS, proved to be a rapid, consistent, and sensitive method for the analysis of sertraline and desmethylsertraline. Analyte peaks were completely resolved, and each provided quantitative and qualitative ions with unique *m/z*. Additionally, no analytes suffered interference from endogenous/exogenous matrix components. The linear dynamic range (LDR) and limit of quantitation (LOQ) for both sertraline and desmethylsertraline were determined, using bovine whole blood as the matrix. The LDR was determined to be 0.78 – 800 ng/mL for sertraline and 1.56 – 800 ng/mL for desmethylsertraline. Illustrated in Figures 2 through 5 are a representative GC/MS chromatogram and mass spectra of sertraline, desmethylsertraline, and SERTIS. The calibration curves for both sertraline and desmethylsertraline had correlation coefficients ≥ 0.99. The LOQ, defined as the lowest detectable analyte concentration that meets all identification criteria (as discussed in the method section), in addition to being within 20% of its target concentration, was determined to be 0.78 and 1.56 ng/mL for sertraline and desmethylsertraline, respectively. The LOD was administratively set at the LOQ.

Analyte carryover was not found to be an issue with GC/MS; however, it was initially investigated and subsequently monitored by the use of ethyl acetate blank injections. The injection of an ethyl acetate blank following a 800 ng/mL blood calibrator showed no carryover contamination. Subsequently, two ethyl acetate blanks were utilized between each postmortem specimen throughout the sample sequence to ensure that no carryover from sample to sample had occurred. Additionally, multiple solvent washes of the injector syringe was carried out prior to and after injection of the sample onto the GC/MS. Any specimen concentration falling outside the LDR was diluted and re-extracted.

Postmortem Concentrations of Sertraline and Desmethylsertraline

Specimens from fatal aviation accident victims are routinely sent to CAMI for toxicological analysis. Postmortem fluid and tissue samples obtained from 11 separate aviation fatalities (years: 2001-2004; ages: 31 – 66; gender: male) that had previously screened positive for sertraline were re-examined using the current method. The fluid and tissue specimens examined from each victim, if available, included: blood, urine, vitreous humor, muscle, liver, kidney, lung, spleen, brain, heart, and bile.

The pharmacology, pharmacokinetics, and pharmacodynamics of sertraline are beyond the scope of this paper. These topics are, however, extensively discussed elsewhere.[1,4,7] Therapeutic blood concentrations for sertraline range from 0.050 to 0.250 µg/mL.[1] Toxic levels of sertraline averaged 0.245 µg/mL in 31 overdose patients.[17] As is evident from these two studies, there is significant overlap in the upper therapeutic and lower toxic levels of sertraline. Lethal levels of sertraline have been reported at concentrations of 1.50 µg/mL and above.[16,18,19] Blood concentrations observed in the current study ranged from 0.005 to 0.392 µg/mL, representing mid-therapeutic to possibly toxic levels. However, since the site from which

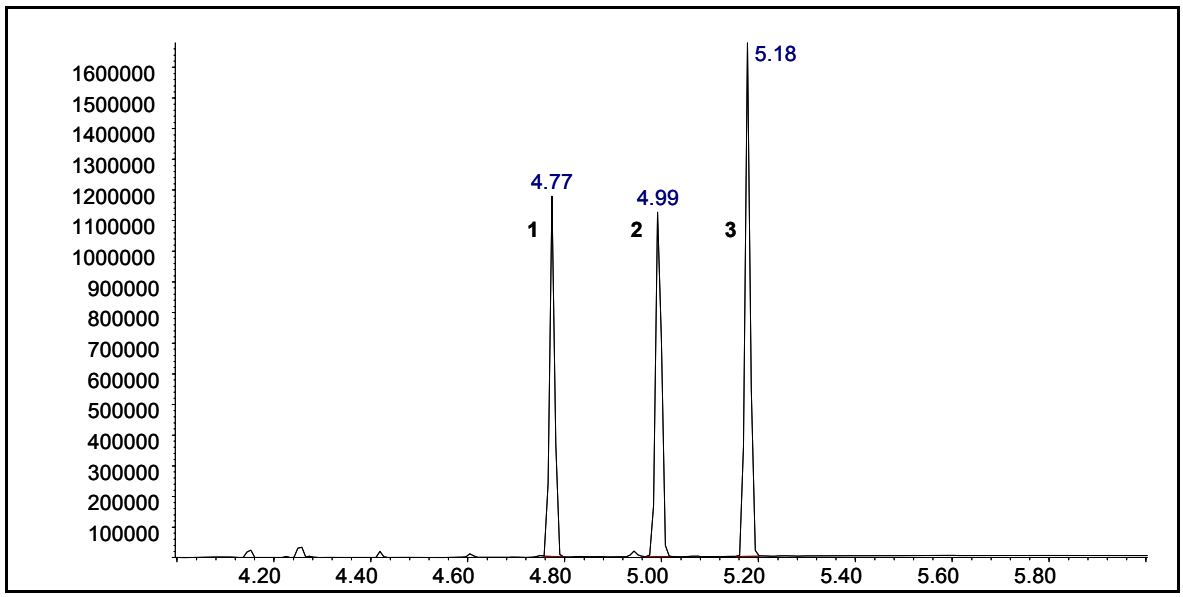

Figure 2. Representative GC/MS chromatogram of 80 ng/mL blood control: (1) desmethylsertraline, (2) internal standard, sertis, and (3) sertraline (all compounds PFPA derivatives).

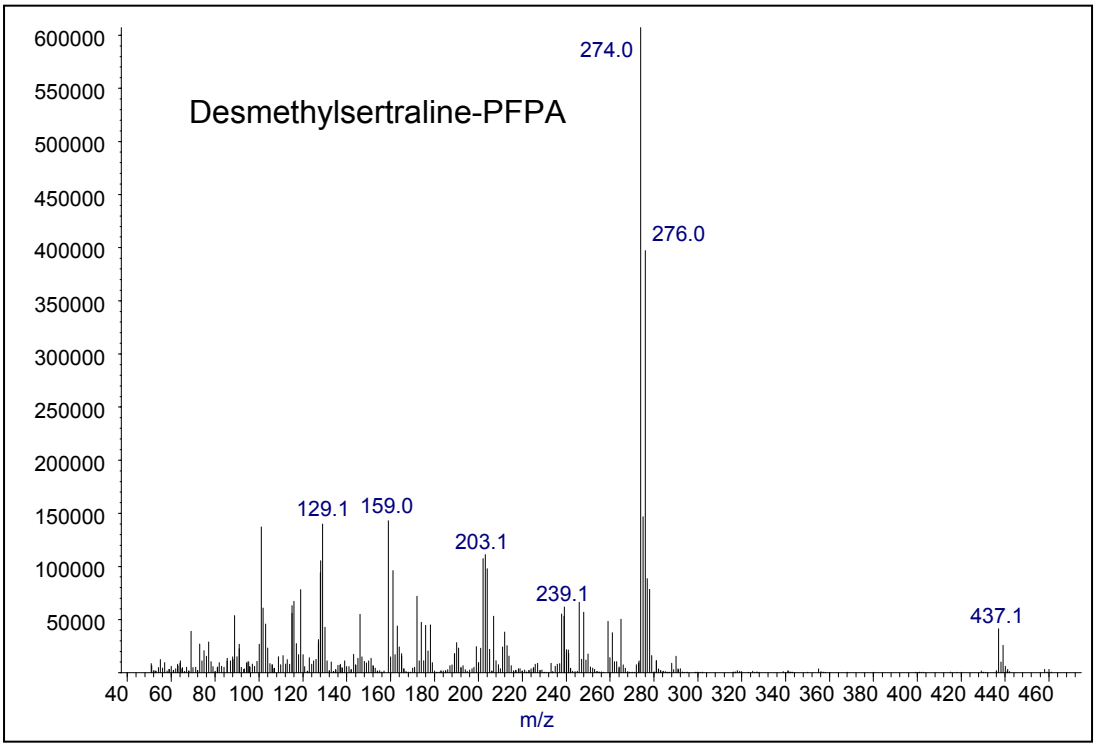

Figure 3. Representative mass spectra for desmethylsertraline (PFPA derivative).

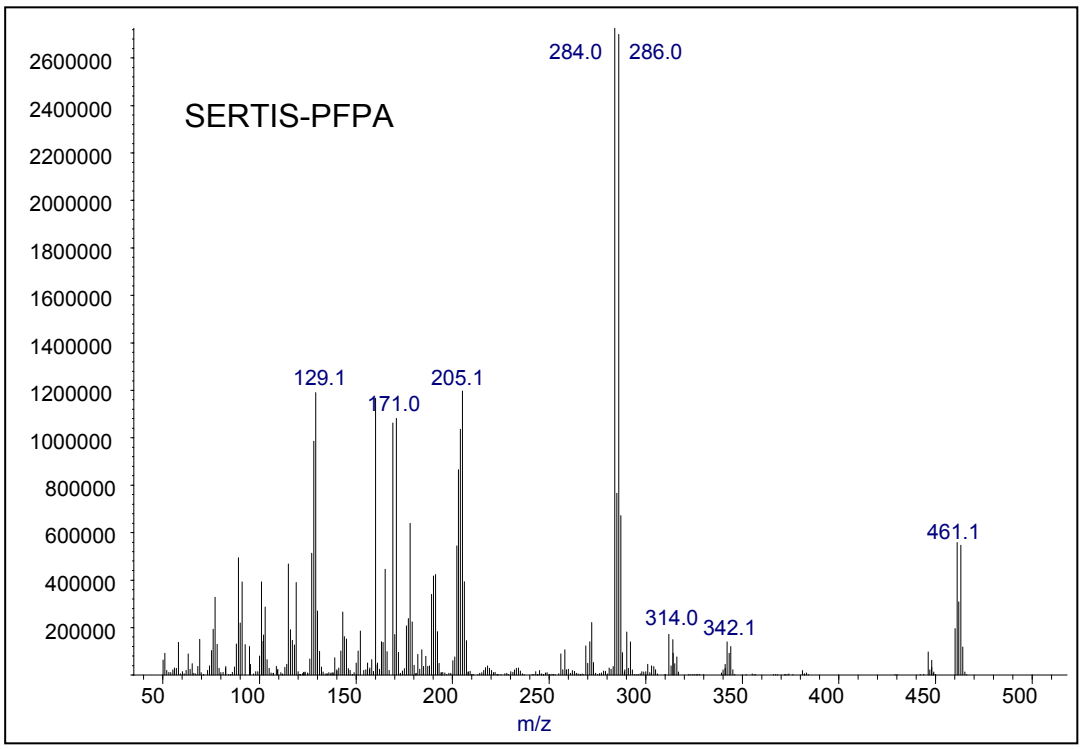

Figure 4. Representative mass spectra for sertis (PFPA derivative).

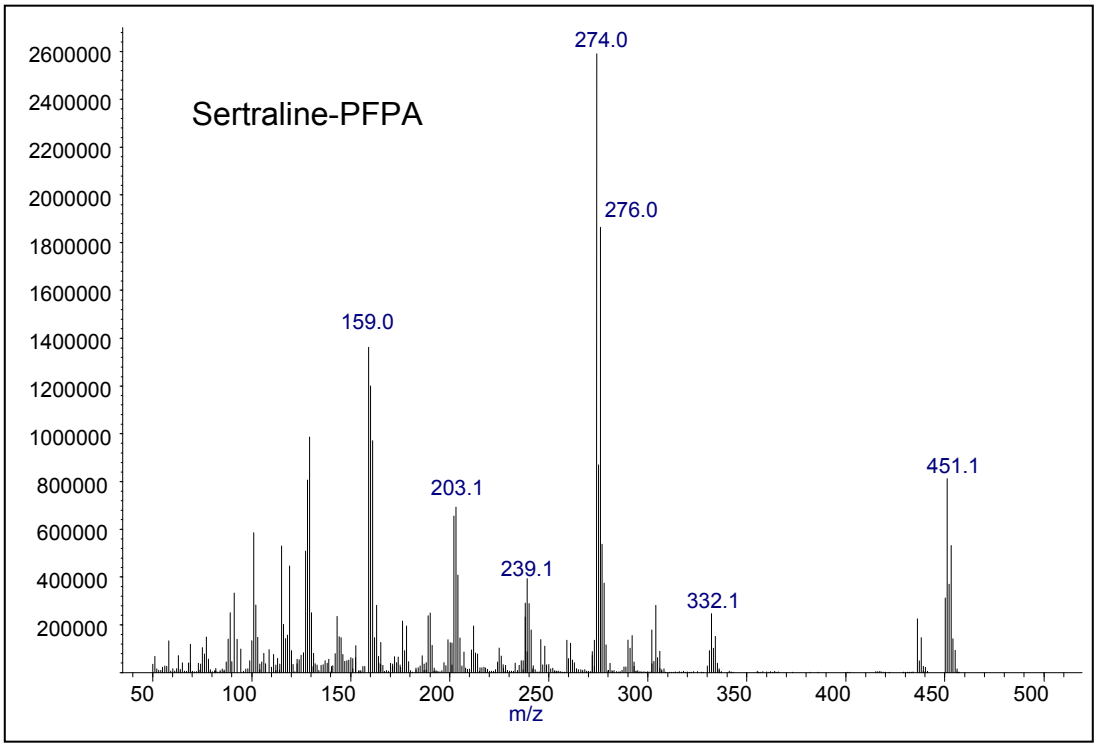

Figure 5. Representative mass spectra for sertraline (PFPA derivative).

the blood was collected at autopsy is unknown for each of these cases, and due to postmortem redistribution (PMR) or other factors, these blood concentrations may not be representative of the levels observed prior to death.[20] The interpretation of quantitative data obtained from specimen types other than blood should be scrutinized due to possible variations in extraction efficiency between specimen types since a non-deuterated internal standard was used. The concentration of sertraline and desmethylsertraline in each postmortem specimen analyzed from these 11 cases are presented in Tables 2 and 3. On average, the highest concentrations of sertraline and desmethylsertraline present in each victim were found in liver and lung tissue specimens. The general trend for highest concentration to lowest concentration of sertraline was: liver, lung, spleen, brain, bile, kidney, heart, muscle, blood, urine, and vitreous humor. With a V_d for sertraline, ranging from 20 to 76 L/kg, we expected concentrations of this analyte to be high in the tissues analyzed. A similar trend holds true for desmethylsertraline (Table 3).

Tremaine et al. and others have shown that sertraline and its metabolites/conjugates undergo urinary, biliary, and fecal excretion.[3,8,9] An intriguing point regarding the distribution of the parent drug and metabolite is that the concentration of sertraline and desmethylsertraline found in urine was lower relative to blood and/or other tissue specimens. Levine et al. and Logan et al. also presented this similar toxicological finding.[5,12] However, these outcomes of sertraline and desmethylsertraline (urine vs. blood) contradict what is typically reported with most antidepressant medications (tricyclic antidepressants, tetracyclic antidepressants, and other SSRIs), where the parent drug and metabolite in urine surpass concentrations detected in the blood following postmortem toxicological investigations.[18,21-24]

Table 2. Sertraline concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	0.022	0.025	—	7.089	2.091	0.130	—	0.078	1.263	0.497	—
2	0.028	—	—	0.349	3.677	0.136	0.195	—	0.584	0.064	—
3	0.302	0.053	0.004	9.640	12.633	1.518	21.477	0.265	5.147	2.192	2.115
4	0.093	—	—	7.078	2.081	0.487	1.457	—	0.929	0.631	4.865
5	0.064	0.053	0.001	6.634	7.028	1.179	3.104	0.224	1.366	1.201	5.009
6	0.043	—	—	4.383	1.003	0.165	7.188	0.065	1.541	0.154	2.918
7	0.240	0.085	0.007	5.255	5.280	0.884	9.161	0.240	2.738	0.383	1.871
8	0.005	—	—	0.232	0.789	0.038	0.111	—	0.08	0.020	0.187
9	0.143	0.004	0.001	3.010	10.767	0.533	1.485	0.129	2.072	0.747	2.380
10	0.036	0.010	—	6.363	2.330	0.618	1.681	0.146	—	0.740	—
11	0.392	—	—	13.517	4.310	2.340	12.226	0.580	4.880	2.069	8.157

***** All concentrations shown in units of µg/mL or µg/g
— Specimen type not available for analysis

Table 3. Desmethylsertraline concentrations obtained from 11 pilot fatalities.*

Case	Blood	Urine	VH	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	0.078	0.119	—	16.569	7.642	0.310	—	0.166	8.542	1.191	—
2	0.011	—	—	0.544	1.457	0.134	0.119	—	0.590	1.491	—
3	0.772	0.090	0.007	16.340	9.488	1.108	54.381	67.470	11.038	2.104	11.615
4	0.071	—	—	4.700	0.780	0.375	0.911	—	1.204	0.174	6.377
5	0.032	0.021	0.002	7.810	8.173	1.178	8.425	0.066	1.377	0.293	5.688
6	0.160	—	—	31.311	1.493	0.424	35.206	0.040	10.270	0.284	15.158
7	0.393	0.167	0.006	17.222	13.678	2.576	6.390	0.277	6.136	1.005	25.188
8	0.028	—	—	2.048	5.637	0.289	0.829	—	0.607	0.199	2.687
9	0.221	0.071	0.002	10.434	13.632	1.253	5.335	0.065	4.915	0.723	6.398
10	0.018	0.034	—	4.491	2.643	0.743	1.774	0.047	—	0.261	—
11	0.348	—	—	18.658	3.246	2.637	19.409	0.292	8.847	0.799	13.491

***** All concentrations shown in units of µg/mL or µg/g
— Specimen type not available for analysis

Table 4. Ratio of desmethylsertraline to sertraline in postmortem specimens.

Case	Blood	Urine	VH*	Liver	Lung	Kidney	Spleen	Muscle	Brain	Heart	Bile
1	3.5	4.8	—	2.3	3.7	2.4	—	2.1	6.8	2.4	—
2	0.4	—	—	1.6	0.4	1.0	0.6	—	1.0	23.3	—
3	2.6	1.7	1.8	1.7	0.8	0.7	2.5	254.6	2.1	1.0	5.5
4	0.8	—	—	0.7	0.4	0.8	0.6	—	1.3	0.3	1.3
5	0.5	0.4	2.0	1.2	1.2	1.0	2.7	0.3	1.0	0.2	1.1
6	3.7	—	—	7.1	1.5	2.6	4.9	0.6	6.7	1.8	5.2
7	1.6	2.0	0.9	3.3	2.6	2.9	0.7	1.2	2.2	2.6	13.5
8	5.6	—	—	8.8	7.1	7.6	7.5	—	7.6	10.0	14.4
9	1.5	17.8	2.0	3.5	1.3	2.4	3.6	0.5	2.4	1.0	2.7
10	0.5	3.4	—	0.7	1.1	1.2	1.1	0.3	—	0.4	—
11	0.9	—	—	1.4	0.8	1.1	1.6	0.5	1.8	0.4	1.7
Mean	2.0	5.0	1.7	2.9	1.9	2.1	2.6	32.5	3.3	3.9	5.7
S.D.	1.6	5.9	0.5	2.6	1.9	1.9	2.1	83.9	2.5	6.7	5.0
CV	82	117	29	88	101	88	82	258	76	170	89

* vitreous humor
S.D. – standard deviation
CV – coefficient of variation
— Specimen type not available for analysis

In a human study conducted by Pfizer, radiolabeled sertraline was orally administered to two healthy male subjects. The percentage of total radioactivity recovered in the urine was 40-45% after 9 days, but no ^{14}C-sertraline itself was identified. However, approximately 40-45% of the radiolabeled dose was sequestered and accounted for through fecal elimination, including 12-14% of unmetabilized sertraline.[3] Furthermore, our laboratory observed increased sertraline and desmethylsertraline levels in bile relative to urine specimens (Tables 2-3).

These results would imply that biliary excretion plays a crucial role in the elimination of sertraline and its metabolites/conjugates. Primary factors determining biliary elimination are: 1) molecular weight, 2) polarity, 3) chemical structure, 4) specialized active transport sites in the hepatic cell membranes, and 5) reabsorption back into the gastrointestinal tract, thereby facilitating enterohepatic cycling.[25] Furthermore, there is a direct correlation between plasma protein binding and the rate of renal filtration of drugs.[26] Since sertraline is highly bound to plasma proteins, these protein complexes may be too big to pass through the pores of the glomeri, thereby inhibiting glomerular filtration. These factors may account for the lower urine levels with respect to blood, bile, and/or other tissue specimens; although additional underlying mechanisms regarding this phenomenon remain unclear.

We evaluated the desmethylsertraline/sertraline ratio within each of the specimen types analyzed, as illustrated in Table 4. In almost every instance, sertraline was at higher concentrations than its metabolite. However, no significant correlation between sertraline and desmethylsertraline concentrations existed within or between any of the specimen types analyzed.

Table 5. Postmortem tissue distribution coefficients for sertraline.

	Urine/ Blood	VH*/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood	Bile/ Blood
n	6	4	11	11	11	10	8	10	11	8
Mean	0.47	0.02	74	67	7.4	46	2.1	22	9	36
S.D.	0.39	0.01	59	45	5	45	1.3	14	7	26
CV	84	47	80	67	68	99	60	65	84	72

* vitreous humor
S.D. – standard deviation
CV – coefficient of variation

Table 6. Postmortem tissue distribution coefficients for desmethylsertraline.

	Urine/ Blood	VH*/ Blood	Liver/ Blood	Lung/ Blood	Kidney/ Blood	Spleen/ Blood	Muscle/ Blood	Brain/ Blood	Heart/ Blood	Bile/ Blood
n	6	4	11	11	11	10	8	10	11	8
Mean	0.83	0.02	115	89	12	80	1.1	39	18	76
S.D.	0.02	0.02	87	83	13	86	0.94	29	38	48
CV	80	87	76	93	108	106	83	74	211	64

* vitreous humor
S.D. – standard deviation
CV – coefficient of variation

The mean distribution coefficients for sertraline and desmethylsertraline, expressed as specimen concentration/blood concentration, are listed in Tables 5 and 6. No consistent distribution patterns between cases were observed. The large coefficient of variations (CV) associated with the distribution coefficients were not completely unexpected, as many unknown variables exist in these cases. The large CVs could result from numerous factors, such as differing blood collection sites at autopsy, postmortem interval, PMR, contamination, hydrolysis, bacterial activity, time between oral sertraline administration and death, sertraline dosage, age of the victim, diet, and health of the victim, i.e., renal and hepatic function.[20,27-29]

The blood collection site(s) and postmortem interval for these cases are unknown. However, in most of the cases we receive for analysis when the collection site is reported, the blood typically is noted as having been collected from the chest cavity. Alkaline compounds readily undergo PMR in the interval between death and specimen collection. This redistribution could account for some of the large CV values obtained. This is consistent with what has been reported by other researchers.[12,14,15]

Drug concentrations determined from a blood specimen can aid in determining impairment and/or cause of death. However, due to the violent nature of aviation accidents, our laboratory receives blood in only approximately 70% of the cases examined. If a distribution coefficient has a relatively small CV, it may be possible, with caution, to use a tissue or fluid distribution coefficient to roughly estimate a blood concentration in cases where blood is not available for analysis. However, the results obtained from our limited number of cases (n=11) show that sertraline blood concentrations cannot be estimated, even crudely, from other tissue/fluid concentrations.

SUMMARY AND CONCLUSION

Our laboratory identifies numerous sertraline-positive cases each year. Possible undesirable side effects associated with this medication are of concern in the aviation community and aerospace industry. With this in mind, we have developed a method for the identification, characterization, and quantitation of sertraline and its metabolite, desmethylsertraline, that is rapid, reliable, and extremely sensitive. By utilizing solid phase extraction, a clean extract was achieved that required minimal time and solvent. A total of 101 tissue and fluid samples from 11 accident victims were analyzed to determine sertraline and desmethylsertraline concentrations. Sertraline concentrations ranged from slightly below therapeutic to levels that have been shown to be toxic in some cases. The results obtained from these cases imply that sertraline is readily absorbed by all tissues and fluids in the body. The coefficient of variation obtained for the calculated distribution coefficients were extraordinarily large, suggesting that these substances likely undergo significant postmortem concentration changes and, therefore, should not be used to estimate sertraline blood concentrations from other tissue/fluid concentrations.

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